Exhibit E

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Reverse transcriptase template switching: a SMART approach for full-length cDNA library construction.

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Abstract

Here, we describe a fast, simple method for constructing full-length cDNA libraries using SMART technology. This novel procedure uses the template-switching activity of Moloney murine leukemia virus (MMLV) reverse transcriptase to synthesize and anchor first-strand cDNA in one step. Following reverse transcription, three cycles of PCR are performed using a modified oligo (dT) primer and an anchor primer to enrich the cDNA population for full-length sequences. Starting with 1 microgram human skeletal muscle poly(A)+ RNA, a cDNA library was constructed that contained 3 x 10(6) independent clones with an average insert size of 2 kb. Sequence analysis of 172 randomly selected clones showed that 77% of cDNA clones corresponding to known genes contained intact open reading frames. The average length of complete open reading frames was 2.4 kb. Furthermore, 86% of the full-length clones retained longer 5' UTR sequences than the longest 5' end deposited in the GenBank database. cDNA libraries generated using this method will be useful for accelerating the collection of mRNA 5' end sequence information, which is currently very limited in GenBank.

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MeSH Terms, Substances